

Revolutionizing Liver Transplantation: A Review on How Machine Perfusion Boosts Outcomes Over Traditional Storage Methods

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Abstract: Background: Static cold storage (SCS) has long been the standard method for liver graft preservation, yet its limitations in maintaining marginal graft viability and mitigating ischemia-reperfusion injury (IRI) have fueled the resurgence of machine perfusion (MP) techniques. **Objective:** This review aims to analyze the evolution, modalities, and outcomes of machine perfusion in liver transplantation compared to traditional SCS, highlighting its role in improving graft utilization, viability assessment, and clinical outcomes. **Methods:** A comprehensive literature synthesis of recent randomized clinical trials, cohort studies, and meta-analyses was conducted to evaluate hypothermic (HMP), hypothermic oxygenated (HOPE/DHOPE), and normothermic (NMP) machine perfusion techniques, including hybrid strategies such as DHOPE-COR-NMP. **Results:** Evidence demonstrates that HMP significantly reduces early allograft dysfunction (EAD), biliary complications, and one-year graft loss compared to SCS. NMP enables real-time functional assessment and therapeutic interventions, expanding the donor pool by safely utilizing extended criteria and DCD grafts. Sequential strategies integrating hypothermic resuscitation with normothermic evaluation show synergistic benefits. Emerging biomarkers such as flavin mononucleotide (FMN), biliary bicarbonate, and transcriptomic signatures enhance viability prediction and decision-making accuracy. **Conclusions:** Machine perfusion represents a paradigm shift from passive preservation to dynamic, personalized graft management. By improving short-term outcomes, enabling viability-based selection, and addressing logistical challenges, MP is poised to become the cornerstone of modern liver transplantation. Future directions include molecular-guided assessment, cost optimization, and broader clinical integration.

Keywords: Liver transplantation; Machine perfusion; Hypothermic oxygenated perfusion (HOPE); Normothermic machine perfusion (NMP).

Introduction

The Evolution of Liver Transplantation and the Organ Shortage Crisis

Liver transplantation has seen significant advancements since its early development, marked by the pioneering work of Thomas E. Starzl, who successfully performed the first series of liver transplants in the 1960s at the University of Colorado. Initially, liver transplantation outcomes were poor due to significant

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immunological challenges, but this dramatically improved with the introduction of cyclosporine in 1979 by the British surgeon Sir. Roy Calne, which, together with advancements in immunosuppressive therapy, surgical methods, and organ preservation, greatly enhanced patient and graft survival (1).

Over the past decades, static cold storage (SCS) has remained the predominant method for preserving donor livers (2), achieving this by lowering metabolic activity to prolong viability outside the body (3). While SCS is effective for preserving healthy grafts over limited preservation times, it falls short in addressing today's organ shortage crisis (4). The growing imbalance between the availability of donor livers and patient needs has necessitated expanding the donor pool to include extended criteria donors (ECD) and donors after circulatory death (DCD) (5). These marginal grafts are particularly vulnerable to severe ischemia-reperfusion injury (IRI) and have a higher likelihood of complications, including primary non-function (PNF), early allograft dysfunction (EAD), and late biliary damage, with these risks being further increased by extended cold ischemia time (CIT) - the interval from graft cross-clamping in the donor until its reperfusion in the recipient (6).

In response to the limitations of SCS, machine perfusion (MP) has resurfaced as a valuable technique for preserving and assessing marginal grafts, leading to greater organ utilization and better patient outcomes (7). Originally investigated over 50 years ago, this technology fell into obscurity by the end of the last century but has recently regained significant attention within the transplant community (8).

The following review provides a detailed examination of the key MP modalities, their protocols, comparative outcomes, and the critical role of viability biomarkers.

Historical Context: From Simple Cooling to Complex Perfusion

The history of organ preservation is one of continuous innovation, beginning in 1812 with Cesar Julien Jean Le Gallois's proposal that tissue

viability could be sustained by replacing the heart's pumping action with continuous artificial circulation supplied with oxygenated blood, and advancing in 1849 to Carl Eduard Loebell's perfusion experiments on isolated pig kidneys, which marked the first practical attempts to maintain organ function outside the body (9).

The modern era of organ preservation started in the late 1960s with Collins et al.'s development of static cold storage (SCS), which quickly became the preferred method for preserving conventional donor organs because of its simplicity and reliability (5). Organs were flushed with a cold preservation solution and then kept immersed at hypothermic temperatures (0-4°C) to suppress cellular metabolism and lower ATP consumption (10).

The evolution of preservation solutions was crucial to the success of SCS, as the original Collins solution, which was high in glucose and potassium, was effective for kidneys but less so for livers and pancreases due to glucose-induced intracellular acidosis, leading Belzer and Jamieson in the mid-1980s to develop the University of Wisconsin solution (UW), which replaced glucose with impermeant sugars like lactobionate and raffinose to prevent cell swelling and added oxygen radical scavengers and a colloid substrate for improved organ protection, UW solution becoming the gold standard in the 1990s, successfully prolonging organ preservation time from approximately 6 hours to 16 hours, thereby contributing to improved transplant outcomes (5,11). Other alternative solutions, such as histidine-tryptophan-ketoglutarate (HTK), Celsior, and Institute Georges Lopez-1 (IGL-1), were subsequently developed (12).

Even with these developments, static cold storage remains limited by ATP depletion and metabolic waste accumulation at cold temperatures, reducing its effectiveness for preserving marginal and high-risk grafts (13). Due to rising organ demand and the resulting poor performance of SCS with marginal grafts, the transplant community has renewed focus on dynamic preservation, bringing modern machine perfusion back as a strategy to actively restore and assess organs ex vivo (14).

Liver Graft Preservation Modalities

Contemporary organ preservation is defined by a spectrum of techniques, each with a distinct mechanism and clinical objective. These modalities, which have largely supplanted SCS as the preferred method for complex cases, can be categorized by their operating temperature and underlying principles.

Static Cold Storage: The Foundational Standard

SCS remains the most widely used and cost-effective method for organ preservation by lowering temperature to slow metabolism and reduce ATP demand, a process linked to the coenzyme Q10 effect, but because metabolic activity is only slowed, not halted, gradual ATP depletion and lactic acid build-up occur, leading upon reperfusion to IRI, which, although manageable in high-quality livers from healthy donors, renders SCS insufficient for marginal livers from ECD due to their increased susceptibility to complications and graft loss (5,15).

Hypothermic Machine Perfusion (HMP): The Resuscitative Approach

HMP is a dynamic preservation method operating at 4–12°C that maintains a hypometabolic state through active, continuous perfusion of a cooled, oxygenated solution to supply metabolic substrates, remove waste, and primarily recondition the graft by mitochondrial restoration (16). Through oxygen delivery at low temperatures, HMP revitalizes cellular energy reserves and repairs mitochondrial function, which helps reduce organ damage and the severity of IRI upon reperfusion (17)

Hypothermic Oxygenated Perfusion (HOPE), a widely adopted HMP technique, introduces oxygenated perfusate into the donor liver via the portal vein, while its other variant, Dual-HOPE (DHOPE), introduces the perfusate through both the portal vein and hepatic artery (18). A 2025 retrospective cohort analysis comparing 183 liver transplants, 90 of which receiving mono-HOPE and 93 receiving DHOPE found no significant difference in one-year patient survival (81.7% in both cases, $p=0.990$) or one-year graft survival (91.2% vs. 93.3%, $p=0.893$), nor in the rates of non-anastomotic biliary strictures (NAS)

(10.96% vs. 8.22%, $p=0.574$), with a median hospital stay of 29 days for both groups, suggesting that the additional cannulation of the hepatic artery may not be a requirement for all hypothermic perfusion protocols (19).

HMP is most often used as an end-ischemic strategy, in which the liver is first preserved by SCS during transport and then perfused for 1–2 hours just before implantation, a brief yet critical period that restores cellular energy, mitigates injury to the parenchyma and biliary tree, and significantly reduces postoperative complications (20). A recent stage 2 prospective clinical trial has shown that prolonged DHOPE (≥ 4 hours) is safe and feasible, allowing preservation times of up to 20 hours at a 10 °C temperature and thereby addressing a major logistical challenge in transplantation by enabling nighttime organ retrievals to align with daytime surgeries, streamlining workflows, and potentially transforming liver transplantation into a semi-elective procedure (21).



Fig.1. Hypothermic Machine Perfusion of an ECD liver graft using the Liver Assist® system (XVIVO) – Image courtesy of the personal collection of St. Spiridon University Hospital Iași, General Surgery and Liver Transplant Clinic

Normothermic Machine Perfusion (NMP): The Viability Assessment Platform

In contrast to the hypometabolic state of HMP, NMP preserves the organ at physiological temperatures (37°C) using a blood-based perfusate with oxygen carriers and nutrients to sustain full metabolic function ex vivo, and although it is more technically and logistically complex, its major advantage lies in enabling

real-time, dynamic assessment of liver viability(22).

By maintaining full metabolic activity, NMP enables the measurement of functional and injury markers such as biliary parameters, perfusate pH, lactate clearance, and hepatocellular enzyme levels, thereby offering an objective, data-driven foundation for transplant decisions that surpasses the subjective judgment or purely morphological assessment commonly relied upon with SCS (23). NMP also offers a unique window for ex vivo therapeutic interventions, such as reducing steatosis, which can further expand the pool of transplantable organs (24). The concept of combining these two modalities, such as a hypothermic phase followed by a normothermic phase, is emerging as a promising strategy to leverage the strengths of both systems: the mitochondrial resuscitation of HMP and the viability assessment of NMP(18).

Comparative Analysis of Preservation Strategies and Clinical Outcomes

HMP vs. SCS

The evidence base for HMP's superiority over SCS is robust, with several studies highlighting significant clinical benefits. A comprehensive meta-analysis of 14 HMP studies, including 4 Randomized Clinical Trials, found that for all donor types, HMP was associated with significant improvements in key outcomes, such as the reduction of the odds for NAS (OR 0.43, 95% CI 0.26-0.70, $P<0.01$), major complications (OR 0.62, 95% CI 0.46–0.84, $P<0.01$) and EAD (OR 0.39, 95% CI 0.30–0.53, $P<0.01$), these benefits being even more pronounced in the subgroup of ECD. Furthermore, the study found that HMP significantly improved both 1 year graft survival (OR 2.31, 95% CI 1.54–3.45, $P<0.01$) and 1 year patient survival rates (OR 1.79, 95% CI 1.15–2.79, $P=0.01$) compared to SCS (15).

Another meta-analysis echoed these findings, concluding that HMP reduced biliary complications (RR 0.74, 95% CI 0.61, 0.91, $P=0.004$), NAS (RR 0.34, 95% CI 0.19, 0.61, $P=0.0003$), EAD (RR 0.54, 95% CI 0.42, 0.68, $P<0.00001$), acute rejection (RR 0.54, 95% CI 0.31, 0.94, $P=0.03$), improved

retransplantation (RR 0.42, 95% CI 0.19, 0.93, $P=0.03$) and 1 year graft loss rates (RR 0.38, 95% CI 0.21, 0.68, $P=0.001$) (25).

The PILOT randomized control trial provided further validation, demonstrating that HMP was noninferior to SCS and was associated with a lower incidence of PNF and biliary strictures (26).

A 2025 retrospective study comparing a small cohort of HMP preserved livers to SCS preserved livers found that HMP was associated with a 100% one-year survival rate compared to 84.4% for SCS. HMP also significantly reduced surgical and biliary complications and improved hemodynamic stability post-reperfusion (27).

NMP vs. SCS

NMP has also demonstrated clear advantages over SCS, primarily in its capacity to expand the donor pool and improve outcomes for high-risk grafts. A 2025 large single-centre observational cohort study of over 1000 transplants found that NMP significantly improved clinical outcomes and reduced hospital resource use, particularly for DCD allografts. The NMP group had a significantly lower EAD rate, with the lowest one being for the DCD NMP group (17.5%) compared to DCD SCS group (50%) ($P<0.01$). The study found a striking 87% decrease in the risk of graft failure for DCD allografts preserved with NMP compared to SCS (HR, 0.13; 95% CI, 0.05-0.33; $P<.001$), and that NMP was significantly protective from patient mortality compared to SCS (HR, 0.31; 95% CI, 0.12-0.80; $P=.02$), highlighting NMP's ability to salvage and optimize organs that would otherwise be at high risk (28).

A cost-effectiveness analysis reinforced this finding, showing that using NMP for discarded organs could raise the annual transplant rate by around 5% and represents a cost-effective approach (\$33,575 per additional quality-adjusted life year), highlighting that NMP's benefits go beyond clinical results, contributing positively to both organ utilization and healthcare economics (29).

Distinguishing HMP from NMP Understanding HMP and NMP in depth

reveals that they are not simply alternative options but interventions with unique mechanisms and applications: HMP operates as a resuscitative method, working to repair mitochondrial injury and restore cellular energy through oxygenation at low temperatures(17,18), while NMP is primarily a diagnostic and therapeutic platform, operating at physiological temperatures to allow for real-time viability assessment and targeted interventions (30).

These differences have significant logistical and financial implications. NMP protocols are

more complex, requiring a blood-based perfusate, which is expensive and logistically demanding due to blood cross-matching and storage requirements (22). HMP, particularly as an end-ischemic strategy, is a more straightforward and "substantially cheaper approach" that does not require a blood-based perfusate (17). Widespread adoption of MP in the United States has been slower than in Europe, with surgeons citing program costs, staff demands, and logistical challenges as key barriers (31).

Table 1. Summary of the key differences between the preservation strategies presented in this review.

	SCS	HMP	NMP
Preservation Temperature	4°C	4–12°C	35–37°C
Primary Mechanism	Passive metabolic suppression ("coenzyme Q10 effect")	Active mitochondrial restoration and metabolic washout	Sustained physiological metabolism and function
Perfusate Composition	Crystalloid solution (UW, HTK)	Crystalloid solution, often oxygenated	Blood-based perfusate with oxygen carrier
Primary Goal	Passive preservation and transport	Active reconditioning and injury mitigation	Viability assessment and therapeutic intervention
Key Clinical Advantage	Simple, low cost, convenient for transport	Reduces biliary complications and EAD, improves survival in high-risk grafts	Allows for objective viability assessment, expands donor pool
Major Limitation	Inadequate for marginal grafts, susceptible to prolonged CIT effects	Limited assessment capability compared to NMP	High cost, logistical complexity, requires specialized personnel

The Synergistic Approach: DHOPE – COR – NMP

This multiphase protocol is intended to facilitate initial resuscitation followed by thorough evaluation of high-risk donor livers that might otherwise be classified as unsuitable for transplantation(32). The perfusion solution employed throughout this protocol often includes a haemoglobin-based oxygen carrier (HBOC), which facilitates effective oxygen delivery across the different temperature phases (33).

The protocol typically begins with 1 hour of DHOPE at a hypothermic temperature (8°C–10°C) to resuscitate the mitochondria and replenish cellular ATP levels, thereby mitigating IRI. This is followed by a 1 hour controlled oxygenated rewarming (COR) phase to gradually bring the liver to normothermic temperature (37°C), preventing sudden temperature shifts that could exacerbate IRI. The final phase involves normothermic machine perfusion (NMP) for a minimum of 2.5 hours at 37°C, allowing for a comprehensive assessment of the liver's viability

based on predefined criteria (34). These criteria typically include the normalization of perfusate pH and lactate levels, bile production of ≥ 10 mL, and a biliary pH > 7.45 within the first 150 minutes of NMP (33).

The rationale behind this sequence is to leverage the benefits of hypothermic perfusion for initial resuscitation and then assess the liver's functional capacity under near-physiological conditions during NMP, with a prospective clinical trial reporting a 20% increase in the number of initially declined high-risk DCD donor livers that were ultimately resuscitated and safely selected for liver transplantation, with 100% patient and graft survival at 3 and 6 months, therefore contributing to a broader inclusion of previously unsuitable livers in the donor pool (34).

A notable downside of the DHOPE-COR-NMP technique is the requirement for the liver graft to be cooled down after the NMP stage and before the surgical anastomosis, as it is stated in a 2025 protocol published by van Leeuwen et al: "Upon cessation of machine perfusion, flush the liver with at least 2L of ice-cold preservation solution, and place it in a bowl with preservation solution with sterile ice" (35). This cooling step introduces an additional logistical consideration and a period of static cold ischemia, although it is likely to be relatively short.

Biomarkers for Liver Viability and Quality Assessment

The ability to objectively assess graft viability is a cornerstone of modern machine perfusion, distinguishing it from the purely descriptive approach of SCS. The data from MP, derived from the perfusate and bile, offers a critical window into the health and function of the organ.

Perfusate and Bile Biomarkers

The bile ducts are a major site of injury following transplantation, often referred to as the "Achilles' heel" of the procedure (36). Damage to cholangiocytes can lead to ischemic-type biliary lesions (ITBL) and NAS, which require costly interventions and can lead to graft failure (7).

NMP provides a unique platform for monitoring bile biochemistry as a surrogate for bile duct

health. Key biomarkers identified during NMP include biliary bicarbonate and pH - healthy bile duct epithelium actively secretes bicarbonate, which maintains a protective, alkalotic pH against toxic bile salts. A biliary bicarbonate concentration greater than 18 mmol/L and a biliary pH greater than 7.48 are strong indicators of low bile duct injury and a low risk of post-transplant cholangiopathy (37).

Biliary Glucose is another biomarker identified during NMP - healthy cholangiocytes actively reabsorb glucose from bile. Ischemia can impair this process, leading to higher glucose levels in the bile. A biliary glucose concentration less than 16 mmol/L and a bile/perfusate glucose ratio less than 0.67 are associated with low bile duct injury (37).

Perfusate analysis during NMP also provides critical information on hepatocellular function. Monitoring perfusate lactate clearance, pH, and hepatocellular enzyme levels (AST) can provide a real-time snapshot of the liver's metabolic state and the extent of cellular injury (30).

Molecular and Genetic Biomarkers

The future of viability assessment is moving beyond macroscopic and biochemical markers to a molecular level. Recent research has focused on identifying genetic signatures that predict graft quality and function. One study identified a 7-gene signature during NMP that corresponded with the benchmarking criteria for transplantation or discard with an area under the curve of 0.99. The specific genes within this signature have been identified as having high predictive value: CD274 gene expression encoding programmed cell-death ligand-1 was found to have the highest predictive value for assessing graft quality and predicting early graft function. The other one is LEAP2 gene expression - at 6 hours of NMP correlated with impaired graft function. This research suggests that the analysis of gene expression markers could serve as a reliable tool to evaluate liver quality during NMP, moving transplant decisions from a subjective to a data-driven, molecularly informed process (38).

Flavin Mononucleotide (FMN): A Promising Marker of Mitochondrial Injury

FMN has emerged as a particularly strong biomarker for assessing liver viability due to its direct link to mitochondrial function and IRI. FMN is a molecule bound to mitochondrial complex I, which is essential for cellular energy production. During periods of ischemia, the bond between FMN and complex I weakens (39). When reperfusion occurs, this can lead to the dissociation of FMN, which results in mitochondrial dysfunction and the generation of reactive oxygen species (ROS). The release of FMN into the perfusate is, therefore, a signature of liver graft injury (40).

Studies have shown that FMN levels in the perfusate are highly predictive of clinical outcomes. A retrospective analysis of samples from multiple centres demonstrated that FMN values measured during HOPE were predictive of graft loss, cholangiopathy, and kidney failure after transplantation. The predictive value of

FMN was found to be superior to conventional donor and recipient risk scores, such as the donor risk index and the balance of risk score (41).

In the context of NMP, FMN has also proven to be a superior biomarker for graft assessment. One study found that perfusate FMN was the best predictor of graft loss compared to traditional viability markers, with a high predictive accuracy. This study established a cut-off value, noting that elevated perfusate FMN of greater than 1.75 µg/mL at 4 hours was independently associated with reduced graft survival. The implementation of FMN-based viability assessment has been shown to reduce complications and improve graft survival, even with high-risk donors. Furthermore, FMN-based decision-making was associated with significant cost reductions per graft for both DBD and DCD livers, demonstrating its value in both clinical and economic terms (42).

Table 2. Summary of the viability markers used in the liver graft assessment

Biomarker	Modality	Value	Clinical Significance
Biliary Bicarbonate	NMP	>18 mmol/L	Low bile duct injury; reflects healthy cholangiocyte function
Biliary pH		>7.48	Low bile duct injury; reflects active bicarbonate secretion
Biliary Glucose		<16 mmol/L	Low bile duct injury; reflects intact glucose reabsorption
Biliary LDH		<3689 U/L	Low bile duct injury
CD274 gene expression		High predictive value for graft quality	Corresponds with transplantation criteria
LEAP2 gene expression		Correlates with impaired function	Indicates a higher risk of early graft dysfunction Export to Sheets

The Preservation Journey: From Organ Retrieval to Implantation

The application of machine perfusion techniques has introduced a new paradigm to the traditional liver transplantation process, creating a dynamic, multi-stage preservation journey.

Donor Liver Retrieval (Deceased Donor)

The procedure is divided into warm and cold dissection according to tissue perfusion timing. In warm dissection, a longitudinal incision is made from the suprasternal notch to the symphysis pubis, and a Balfour retractor provides adequate exposure of the abdomen. The round ligament is ligated, the falciform ligament is dissected, and

the abdominal organs are inspected to rule out malignancy, with a liver biopsy taken if necessary. The sternum is opened and divided with an electric saw, followed by placement of a sternal retractor, while the pleura and pericardium remain intact. Retroperitoneal dissection along the white line of Toldt allows mobilization of the ascending colon, identification and preservation of the right ureter, and exposure of the inferior vena cava (IVC), aorta, left renal vein, and superior mesenteric artery (SMA) origin. The inferior mesenteric artery may be ligated for better aortic exposure, and distal and proximal aortic ties are positioned for perfusion and cannula fixation. The inferior mesenteric vein can also be isolated for portal system perfusion if needed. Liver mobilization begins with division of the left triangular and hepato-gastric ligaments, taking care to preserve any accessory hepatic arteries. The common bile duct is dissected and transected, the portal vein visualized, and potential variations of the right hepatic artery checked. The right gastric vessels are tied and divided, while the proper hepatic and gastroduodenal arteries are carefully dissected toward the celiac trunk, with partial dissection of the splenic and left gastric arteries and ligation of the left gastric vein. The hepato-esophageal ligament is divided, the esophagus retracted laterally, and the aorta freed with a nylon tie for subsequent perfusion. The gallbladder is flushed with saline to prevent bile-related autolysis. After cardiothoracic dissection, intravenous heparin is given, the distal aorta is ligated, and a cannula is inserted for cold perfusion, optionally including portal flush via the inferior mesenteric vein. The IVC is opened for drainage, slush ice applied to surrounding organs, and 10–20 L of HTK solution used to maintain organ preservation. Cold dissection typically follows heart and lung procurement, with the liver taken first if the small intestine is not needed. The gastroduodenal artery is ligated, and the portal vein divided depending on whether the pancreas is also being procured. The splenic and left gastric arteries are transected near their origin, while accessory hepatic arteries are preserved when present. Lymphatic and nerve tissues around the celiac trunk are dissected, and the aorta is exposed. Division of the celiac trunk

includes an aortic patch, and the SMA is included in the patch if right accessory or replacement hepatic arteries exist. Finally, the anterior IVC wall is transected above the renal vein. Close coordination between the liver and pancreas teams ensures successful organ retrieval without compromising vascular integrity. During procurement, the iliac arteries and veins are carefully harvested as far distally as the external iliac vessels, typically removed together as a single bundle. Care must be taken to avoid excessive tension on the vessels during extraction. Once harvested, the vessels are submerged in HTK solution and double- or triple-bagged in sterile packaging for safe transport, similar to organ preservation protocols. These iliac vessels serve as excellent conduits for arterial or venous reconstruction during liver and pancreas transplantation. Usually, one set of iliac vessels accompanies the liver graft, while a second set is allocated for the pancreas.

For liver graft packaging and transport, the liver is first inspected and placed into a sterile bag containing 700–1,000 mL of cold (4°C) HTK solution, which is then securely tied. This bag is placed into a second sterile bag filled with 1 liter of cold normal saline or slush ice and tied, followed by a third bag for additional protection. The triple-bagged liver is then positioned in a heat-insulated container surrounded by ice blocks to maintain low temperature during transport, ensuring optimal graft preservation (43).

Machine Perfusion and Organ Preservation

After liver procurement, the “back-to-base” combined hypothermic and normothermic machine perfusion approach involves a sequential protocol designed to minimize ischemic injury and enable functional assessment prior to transplantation. Upon arrival at the perfusion facility, the machine is prepared with a dual-circuit system and primed with approximately 3 L of sterile University of Wisconsin machine perfusion solution, maintaining arterial and portal pressures at roughly 25 mmHg and 5 mmHg respectively, with the temperature kept between 8–12 °C. Oxygenation during this hypothermic phase (HOPE) is achieved using 100% oxygen at a flow

of 1 L/min, and perfusate oxygen tension is verified to exceed 100 kPa. Initially, perfusion may be applied to the portal vein alone, with subsequent inclusion of the hepatic artery (DHOPE) once cannulation is complete. The hypothermic period should last at least one hour, though it can be extended to accommodate logistics without compromising viability. During the final part of DHOPE, preparations are made for the transition to controlled oxygenated rewarming (COR) and normothermic machine perfusion (NMP). This includes assembling a red blood cell-based perfusate, pharmaceutical additives, colloid and protein solutions, and flushing supplies. The transition requires a rapid “fluid switch,” during which the liver is temporarily immersed in cold preservation solution, the perfusion circuit is rinsed, and the new perfusate is introduced. Before reconnecting, the graft is flushed with cold Ringer’s lactate to remove residual high-potassium solution. COR begins at approximately 20 °C to avoid haemolysis, with gradual temperature increases of about 3 °C every 7–8 minutes until normothermia (37 °C) is reached. Perfusion pressures are adjusted in stages to achieve a portal flow near 90–110 mL/min per 100 g liver weight and an arterial flow of roughly one-third that value. Gas exchange parameters are carefully controlled to maintain an arterial oxygen tension of 10–13 kPa, a venous saturation of 55–75%, and an arterial saturation close to 100%, while preventing excessive CO₂ loss. Once at normothermia, the liver undergoes functional assessment over a 150-minute period. Viability criteria generally include clearance of lactate to below 1.7 mmol/L, maintenance of physiological perfusate pH, adequate bile production (≥ 10 g total and at least 4 g in the last hour), and favourable bile biochemistry, such as a bile pH above 7.45 and a bicarbonate concentration at least 5 mmol/L higher than the perfusate. Arterial, venous, and bile samples are collected at regular intervals to monitor these parameters, and adjustments in oxygen delivery, electrolyte balance, and buffering are made as required. If all viability thresholds are met, normothermic perfusion is continued until the recipient operation is ready to proceed. At the end of

machine perfusion, the graft is flushed with cold preservation solution and stored on ice until implantation. This structured sequence—from end-ischemic dual hypothermic perfusion, through controlled rewarming, to normothermic assessment—combines the protective effects of cold oxygenation with the physiological advantages of warm perfusion, allowing both recovery and real-time evaluation of marginal or extended-criteria livers before transplantation (35).

Recipient Hepatectomy and Graft Implantation

The recipient is prepared on a separate table once the graft is available. A large right subcostal modified Makuuchi (“hockey stick” or “reverse L”) incision is typically used, providing exposure of the liver and the suprahepatic IVC. The Mercedes incision may be used in special occasions such as obesity or adhesions, but at a higher risk of developing incisional hernias, and in some selected cases, a supra-umbilical median incision can be used. After laparotomy, the falciform ligament is divided and the liver is mobilized. The left triangular and coronary ligaments are dissected first, freeing the left lobe. Next the gastrohepatic ligament is opened and examined for any arterial anatomical variation. The ligamentum teres is divided. In cirrhotic recipients, traction can tear the capsule, so excessive force should be avoided. Instead the right lobe may be mobilized early and a large piece of gauze can be placed behind the liver to bring the hilum anteriorly for better exposure, facilitating the dissection of the hepatoduodenal ligament (44).

After the hilum is addressed, any remaining suspensory attachments – including the divided falciform, left triangular and coronary ligaments – are cut, and the bare areas on the left and right are entered so that the right lobe can be elevated and the supra- and infrahepatic cava exposed. In cases of massive portal hypertension or dense hilar adhesions, the entire hilum may be encircled early and clamped in bloc, then individual structures (hepatic artery, bile duct, portal vein) divided inside the clamp to control bleeding. (45)

. In selected cases, such as patients with

portal hypertension, an intraoperative venovenous bypass (VVB) may be used, by placing cannulas in a femoral vein and the portal vein, creating a shunt that maintains venous return, helps with keeping the hemodynamic stable during the clamping of the vena cava and portal vein, resulting in decreased bowel edema and renal congestion (46).

After the hepatectomy, meticulous haemostasis is achieved and the bare area hepatic surfaces are oversewn, the caval cuffs are then prepared for anastomosis. The infrahepatic IVC cuff is fashioned simply by trimming the remaining recipient cava, small tears are carefully oversewn before revascularization. The suprahepatic cuff is created by opening the septa between the cut hepatic veins to form a single wide funnel-shaped lumen. The donor liver is brought into the field, and an end-to-end suprahepatic IVC anastomosis is performed, using continuous non-absorbable sutures, between the recipient's funnel cuff and the donor cava. Next, the infrahepatic IVC is anastomosed in a similar fashion. During the infrahepatic suture line, the graft is flushed with cold solution via the portal cannula to wash out preservation fluid. The portal cannula is removed, and the donor and recipient portal veins are trimmed to equal length and sewn end-to-end. Care is taken to avoid an excessively redundant segment. After unclamping the portal vein, flow into the graft is confirmed. The hepatic artery is then reconstructed. The recipient artery is dissected up to a healthy segment (often near the common or proper hepatic artery) and an end-to-end arterial anastomosis is fashioned with fine running sutures (typically 6-0 or 7-0 polypropylene) under magnification. If the arteries are too short or mismatched, an interposition graft (usually from the donor iliac artery) may be used to reach the recipient vessel. Once arterial flow is confirmed (with Doppler), attention turns to bile drainage. The bile duct is then reconstructed – in adults usually by duct-to-duct anastomosis over a stented T-tube if the recipient duct is healthy, or by a Roux-en-Y choledochojejunostomy if needed. The entire reconstruction is tested (with Doppler or cholangiography) to ensure good flow. With all vascular and biliary anastomoses

complete, haemostasis is finalized, drains are placed, and the abdomen is closed (45).

Conclusion

The data from numerous clinical trials and meta-analyses overwhelmingly confirms that machine perfusion, particularly HMP, is a safe and effective preservation strategy that significantly improves clinical outcomes compared to SCS, especially for DCD and ECD livers (15,25,47). This paradigm shift from passive preservation to an active, dynamic approach is fundamentally reshaping the transplant landscape.zA

The evolution of machine perfusion is moving beyond a simple technological fix to a holistic system that addresses not only biological challenges but also logistical and economic ones. The development of prolonged DHOPE protocols, for instance, allows for extended preservation times, providing a critical logistical advantage by enabling nighttime organ retrievals to be matched with daytime surgeries (21). This can improve the work-life balance and safety of the surgical team, which is an important, non-clinical factor in the adoption of new technologies.

Despite the clear benefits, widespread adoption of MP, particularly NMP, has been slower in some regions due to multi-factorial reasons. Key barriers cited by transplant surgeons include high program costs, the demand for specialized personnel, and logistical complexities. Future efforts must focus on refining cost-effectiveness analyses, collaborating with device companies and organ procurement organizations, and developing standardized training protocols to overcome these challenges and maximize the clinical impact of MP on liver transplantation (31).

A highly promising future direction is the use of a combined perfusion strategy, leveraging the distinct strengths of HMP and NMP in a sequential manner. A model where HMP is used for initial mitochondrial resuscitation, followed by NMP for viability assessment and targeted therapeutic intervention, holds the potential to "dramatically increase" the pool of usable livers

and improve outcomes for recipients(8). The refinement of molecular biomarkers, such as gene expression signatures, will further enhance this personalized, data-driven approach to graft selection, providing a high degree of confidence that a marginal liver can be safely transplanted (38).

NOTES

*All authors contributed equally to this work.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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